A METHOD OF ISOLATING THE PNEUMOCOCCUS IN MIXED CULTURES, SUCH AS THROAT CULTURES.*

GUSTAV F. RUEDIGER.

(From the Memorial Institute for Infectious Diseases, Chicago.)

ABOUT a year ago Hiss[1] published a paper in which he recommended inulin as an aid in differentiating the pneumococcus from the streptococcus. He showed that pneumococci ferment inulin, when added to a sugar-free culture medium which is favorable for the development of these organisms, while streptococci fail to ferment it. This work has since been taken up by several investigators in this country, and it has been found that Hiss's observations are correct, with perhaps one exception. There is a group of organisms[2] which appear to be identical with the pneumococcus, both morphologically and culturally, but they do not ferment inulin. In spite of this fact, however, it must be stated that the inulin reaction is often a convenient method of determining to what class a certain organism belongs.

A number of methods are in use for isolating pneumococci from the mouth in health and in disease. One of these is by animal inoculation. Usually a rabbit or a white mouse is inoculated with a quantity of sputum, and if the animal dies the blood and internal organs are examined for encapsulated diplococci. If the animal does not die, the experiment does not show much, because pneumococci may have been present, but in too small a number, or their virulence may have been too low to produce death. Various plate methods are also in use, but of these I shall mention only the blood-agar plate method. It is well known that in this medium *Streptococcus pyogenes* forms small gray colonies, which are surrounded by a zone of hemolysis, while the pneumococcus and *Streptococcus viridans* of Schottmüller[3] form green colonies. To isolate pneumococci from blood-agar plates which have been inoculated with material from throats, it is necessary to make subcultures from at least 10 to 15

*Received for publication January 25, 1906.

[1] *Jour. Exper. Med.*, 1905, 7, p. 317.

[2] See PARK AND WILLIAMS, *Jour. Exper. Med.*, 1905, 7, p. 403; also DUVAL AND LEWIS, *ibid.*, p. 473.

[3] *Münch. med. Wchnschr.*, 1903, 50, pp. 849 and 909.

green colonies. In this way I hardly ever failed to get at least one culture that ferments inulin, and from some throats I have obtained as high as eight inulin fermenters out of ten cultures tested. Although this method gives fairly satisfactory results, it involves a large amount of useless labor. With the object of making the isolation of pneumococci easier, I have prepared a blue litmus inulin-agar medium, in which pneumococci form red colonies. This medium is simply a sugar-free agar, with the addition of inulin and litmus, and is made as follows:

(*a*) Peptone (Witte)	10
Agar-agar	15
Sugar-free beef broth (neutral)	1,000

Dissolve by boiling one hour, adding water from time to time to make up the loss from evaporation. Heat in the autoclav for 15–20 minutes (to prevent subsequent precipitation while sterilizing), clarify with egg, filter through cotton, and make the volume up to 800 c.c. with distilled water.

(*b*) Dissolve 15 grams of pure inulin in 200 c.c. of distilled water, mix this solution with (*a*), add 20 c.c. of a 5 per cent solution of litmus (Merck's highest purity), tube, and sterilize in the autoclav under 10 pounds pressure for 15 minutes. Each tube should contain 7 or 8 c.c. of medium. Some pneumococci do not grow well in this medium, hence it is necessary to add 1 c.c. of heated (65° C.) ascites fluid to each tube of melted agar (which has been cooled to 45°) immediately before using.

In this mixture the pneumococci grow very well, and in 24 to 96 hours the inulin fermenters form red colonies (acid production), which stand out very prominently on the blue background. The surface colonies do not produce as much acid as the deep colonies, and it is for this reason that each tube should contain a rather large amount of medium. In this way a thick plate is formed, with relatively few surface colonies.

The pneumococci are practically the only mouth bacteria that ferment inulin. I have tested four strains of *Staphylococcus aureus*, 20 strains of *B. pseudodiphtheriticus*, 120 strains of *Streptococcus pyogenes*, one strain of *Micrococcus catarrhalis*, one strain of *B. mucosus*, and one strain of *Micrococcus tetragenus;* not one of these fermented inulin. Among 10 strains of diphtheria bacilli, I encountered one which ferments inulin. I have studied in detail 22 cultures, made from red colonies in plates of this medium which had been inoculated with material from the throats of five pneumonia patients, six scarlet-fever patients, and one case of pharyngitis. All of these

organisms are Gram-positive, lanceolate, oval, or rounded cocci, which grow chiefly in pairs on blood agar and serum agar, but are also found in short chains in liquid media. All of them ferment inulin,* and all but four form green colonies in blood-agar plates. Capsules could be demonstrated on 13 cultures, while two were doubtful. The details are shown in the accompanying table:

TABLE 1.

SHOWING THE CHARACTERISTICS OF ORGANISMS ISOLATED FROM THROATS BY MEANS OF LITMUS INULIN-AGAR PLATES.

Organism	Source	Morphology		Colonies in Blood-Agar Plates	Capsules
		On Blood-Agar Slants	In Calcium Broth		
1	Pneumononia Throats	Round and oval cocci in pairs and chains	Diplococci		+
1a		Lanceolate diplococci	Diplococci	Green	−
2		Lanceolate diplococci and short chains.	Diplococci and chains	Slightly hemolyzing. Not green	+
2a		Lanceolate diplococcus and short chains	Diplococci	Small, brownish	−
3		Lanceolate diplococci	Short chains and diplococci	Green	+
3a		Diplococci	Diplococci	Small, brownish	−
4		Lanceolate and round cocci in pairs	Diplococci and short chains	Green	+
4a		Lanceolate and round cocci in pairs	Diplococci and short chains	Green	−
5		Round diplococci	Short chains and diplococci	Green	?
5a		Chiefly short chains. Some diplococci	Short chains and clumps	Slightly hemolyzing	−
6	Scarlet-Fever Throats.	Diplococci	Diplococci	Green	+
7		Lanceolate diplococci	Diplococci	Green	+
7a		Lanceolate diplococci	Diplococci	Green	+
7b		Lanceolate diplococci	Diplococci	Green	+
8		Lanceolate and round cocci in pairs	Short chains and diplococci	Green	+
9		Lanceolate diplococci and short chains.	Diplococci	Green	+
9a		Lanceolate diplococci	Diplococci	Green	+
10		Lanceolate diplococci	Diplococci	Green	−
10a		Lanceolate diplococci	Diplococci and short chains	Green	+
11		Lanceolate diplococci	Diplococci	Green	−
11a		Lanceolate diplococci	Chains	Green	?
12	Pharyngitis	Lanceolate diplococci	Chains and diplococci	Green	−

Some pneumococci isolated by this method are somewhat atypical in that they tend to form chains and have no capsules. Fourteen

*The inulin-fermenting power was tested in the following medium; (*a*) Take 300 c.c. of rich, sterile ascites fluid, add 100 c.c. of sterile distilled water, and heat to 65° to 70° for one-half hour. (*b*) Dissolve 6 grams of inulin and 6 grams of peptone (Witte) in 200 c.c. of distilled water, add 6 c.c. of a 5 per cent litmus solution, and sterilize in the autoclave under 10 pounds pressure for 15 minutes. Mix (*a*) with (*b*), tube under aseptic precautions, and incubate the tubes 24 hours before using. This medium is very favorable for the growth of pneumococci, but care must be taken not to use ascites fluid that contains fermentable carbohydrates.

rabbits were inoculated with large doses of these organisms but only five of the animals died. In the animal body these orgamisms grew almost exclusively in pairs and three of the five strains produced capsules.

In routine examination of throats for pneumococci the following scheme has been found very satisfactory. A sterile cotton swab is rubbed against the tonsils and walls of the pharynx. The swab is rinsed in 1 c.c. of sterile broth, and four or five tubes of litmus inulin-agar are inoculated with this broth and plated. The plates are incubated, and examined for red colonies in 24 hours. If no red colonies are found, the plates must be examined daily for four days, as some pneumococci ferment inulin rather slowly, and red colonies may appear as late as the fourth day.